

Intramuscular Lactate and High Intensity Exercise in Equine Athletes: A Review

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September 2002

Track racing relies heavily on the horse's anaerobic metabolism to produce the speeds necessary to win. One widely accepted theory is that a major limiting factor to high intensity exercise is the production of lactic acid in the muscles. This paper examines the way lactate is produced and utilised in the horse. It outlines how lactate affects the horse during exercise and describes metabolic changes occurring with high intensity exercise.

INTRODUCTION

Lactic acid consists of a hydrogen ion, H^+ , combined with a lactate anion, Lac^- . Lactic acid accounts for more than 99% of the lactate present in the body fluids. Lactate is a quantitatively important organic anion with a dissociation constant (pK) of about 3.7; thus it is mostly dissociated at physiologic intracellular pH values. There has been no upper limit placed on the accumulation rate of lactate (Sejersted 1992) and Hultman and Sjöholm (1986) reported intracellular accumulation of lactate to be as high as 40 mmol/kg wet muscle weight with a concentration of lactate in the cytosol of about 80 mmol/L. With intact circulation, peak Lac^- concentrations have been in the range of 20 to 30 mmol/kg wet muscle weight (Sejersted 1992).

Lactate is produced with the increasing energy requirements during high intensity exercise. Acidosis in muscle during exercise has been attributed to increased lactate concentrations, mainly due to the apparent correlation between lactate production and imbalances between oxygen supply and demand (Jones and Heigenhauser 1992). However, evidence of hypoxia has not been shown with increases in lactate concentration [Lac^-], even during intense exercise (Connett et al. 1986; Stainsby et al. 1989). There are a number of contributing factors to the intracellular hydrogen concentration ($[H^+]_i$) and acid-base balance in muscle (Cheema-Dhadli et al. 2002), including glycolysis and ATP hydrolysis (Hochachka and Mommsen 1983).

Equine muscle pH ranges from 6.2 to 7.4 or greater (Sejersted 1992). The low pH measured in horses at the end of exercise may be an indication of their high glycolytic capacity (Hyypä et al. 1988). In humans, high production of lactate has been associated with superior athletic ability, as a greater capacity to produce and remove lactate from the muscle delays the onset of acidosis (Hyypä et al. 1988; Mannion et

al.1995). Lactic acid is produced in the muscle in increasing quantities as a result of intense exercise and then is utilised as a metabolic intermediate that may be beneficial to the continuation of high intensity activity (Nielsen et al. 2001; Westerblad et al. 2002). The physicochemical approach to acid-base balance outlines how Lac^- and H^+ s are affected by those variables. Consideration of the important factors determining the $[H^+]_i$ will likely have an impact on the training protocols in horse racing (von Duvillard 2001).

ENERGY SYSTEMS

Muscular contraction creates the movement for exercise. The energy requirement for movement is created by the activation of myosin ATPase, breaking down adenosine triphosphate (ATP), and enabling the actin-myosin cross-bridge to develop tension and contract the muscle. ATP is the only fuel for muscular contraction and its turnover is critical in controlling all the subsequent metabolic steps in energy production (Jones and Heigenhauser 1992). The breakdown of ATP initiates a series of biochemical events activating the metabolic energy pathways.

During a horse race, with the oxidative metabolism unable to supply sufficient immediate energy, the demand for ATP is greater than the aerobic energy pathways can provide. When high intensity activities increase the body's energy requirements beyond the ability of the aerobic system to meet the ATP energy demand the anaerobic energy provision increases in proportion to exercise intensity producing Lac^- at increasing rates (Spriet 2000). Energy production pathways within the aerobic system are slow to increase, taking two to three minutes to fully activate. In contrast, the anaerobic pathways are utilised immediately and may be fully activated within the first few seconds with energy provided from the high-energy phosphates, ATP and CP, stored within the

muscle. They provide the initial energy and last in an all out effort for the initial seconds before anaerobic glycolysis becomes fully activated (Bergstrom et al. 1971). Anaerobic energy pathways are thus used in transition from rest to exercise and for short duration, high intensity activity (Jones and Heigenhauser 1992) and anaerobic energy production during glycolysis is important for the maintenance of high intensity exercise (Hyypä et al. 1988; Hargreaves 1995).

The quarter horse derives about 60% of the total energy from anaerobic metabolism during a 400-m race (Eaton 1994). Estimates of the total energy requirements being derived from the anaerobic metabolism for thoroughbreds during a 1,000-m race are up to 70%. During the longer thoroughbred and standardbred races the estimates of energy derived from anaerobic metabolism are up to 55% (Bayly 1985; Eaton 1994). Harris and Snow (1988) measured plasma lactate concentration ($[\text{Lac}]_p$) in horses at various gaits. At a walk they found no significant increase, at a 8 m/s trot they found a small but significant increase in $[\text{Lac}]_p$ and again at 10 m/s. With the onset of cantering or galloping at 12 m/s they found an abrupt increase in Lac^- accumulation (Hyypä et al. 1988).

ATP Turnover

ATP, ADP and P_i are all weak acids. The pK of ATP^{4-} is 6.79, ADP^{3-} is 6.75, and P_i^{2-} is 6.78. Therefore the breakdown of ATP causes minimal changes in H^+ production as the changes in the total concentrations of the reactants are small (Jones and Heigenhauser 1992):



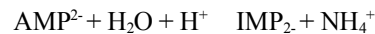
AMP Kinase catalyses the reaction where the ADP, which was generated with the immediate breakdown of ATP, is then immediately available to reform back into ATP:



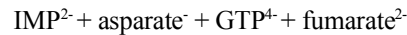
Metabolic processes generate the energy required for muscle activity but an insufficient amount of $[\text{ATP}]_i$ is not believed to cause fatigue (Brooks et al. 2000). Despite large fluctuations in energy demand, with a more than 100 fold increase from to high intensity exercise, muscle ATP levels remain practically constant (Sahlin et al. 1998). Limitations in ATP hydrolysis may be a limit to the energetic processes. The maximal rate of energy expenditure cannot exceed the activity of the ATPase activity (Sahlin et al. 1998).

ATP breakdown during maximal exercise is associated with increases in both ADP and AMP. These phosphates increase adenine nucleotide degradation to inosine monophosphate (IMP), with a resultant

creation of ammonia. Adenylate deaminase catalyses the reaction:

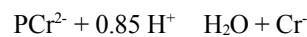


The IMP may then be converted back to AMP by the transfer of nitrogen from aspartate through adenine nucleotide cycle (Lowenstein and Goodman 1978):

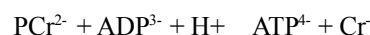


The reactions may help to minimise the $[\text{H}^+]_i$ changes as the optimal pH for the reactions lie between 6.2 and 6.5. An increase in $[\text{H}^+]_i$ could theoretically influence the physiological effectiveness of the purine nucleotide cycle. NH_4^+ , with a pK of 9.4, acts as a strong base, however, it seldom reaches a concentration that can exert an influence on $[\text{H}^+]_i$ (Tullson and Terjung 1991).

Another readily available energy source is available through stored phosphocreatine (PCr^{2-}), which breaks down to creatine (Cr) and inorganic phosphate (P_i^{2-}). The amount of energy produced from PCr^{2-} is limited by its small amount of intramuscular stores (Sahlin et al. 1998). There are also high initial breakdown rates in PCr^{2-} with a greater amount of ATP required for energy in high intensity exercise along with a decrease in intramuscular hydrogen ion concentration ($[\text{H}^+]_i$) as H^+ s are consumed in the reaction (Lindinger 1995):



Both the reactions breaking down ATP^{4-} and PCr^{2-} occur simultaneously as long as each is available. They can be combined to form the reaction catalysed by Creatine Kinase (Lindinger 1995, simplified from Hochachka and Mommansen 1983):



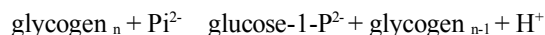
Hultman and Sjöholm (1986) have shown that during exercise there is a linear relationship between the power output and the decrease in $[\text{PCr}^{2-}]_i$, and others have shown a rapid breakdown during maximal exercise (Jones et al. 1985; Heigenhauser et al. 1990). The pK of PCr^{2-} is 4.5, and as a strong acid the major influence of this reaction is through the reduction of $[\text{PCr}^{2-}]_i$, reducing the $[\text{H}^+]_i$ and creating a strong alkalinising effect in muscle during the initial increase in energy requirements (Hultman and Sjöholm 1986).

With low to moderate intensity exercise after the rest to exercise transition, PCr^{2-} is resynthesized in the muscles and the alkalinising effect is negated (Jones and Heigenhauser 1992). At high exercise intensities PCr^{2-} may not be restored until after the exercise has ended (Harris et al. 1976; Sahlin 1978), and thus the

alkalinising effect of PCr^{2-} persists throughout the exercise. This alkalinising effect may be beneficial for the continuation of brief bouts of high intensity exercise (Lindinger 1995).

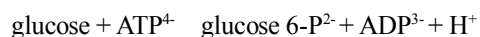
Glycogenolysis and Glycolysis

Glycogenolysis, the breakdown of glycogen to glucose, is activated very quickly following the onset of exercise. Its activation related to the frequency of contractions (Richter et al. 1982). Phosphorylase is the key rate limiting enzyme in both glycogenolysis and glycogen phosphorylase (GP) catalyses the glycogenolysis reaction:



Anaerobic glycolysis takes glucose, a six-carbon sugar, and breaks it down into two three-carbon molecules of pyruvic acid within the cytoplasm creating energy and producing pyruvate, the excess of which is converted to lactate. Within a second of initiating the activity twenty percent of the total energy gained during anaerobic glycolysis is developed and a further 50% is created between 1.25 and 2.5 seconds (Spriet et al. 1987). The rate of glycogenolysis is closely related to the rate of ATP turnover (Ren et al. 1990). The pathway's activation is extremely rapid, within 10 seconds of the contraction being initiated the $[\text{Lac}^-]$ has increased (Jacobs et al. 1985). After the initial 30 seconds, the muscle glycogen content continues to fall by 20-30 mmol/L and $[\text{Lac}^-]$ increases by 20-40 mmol/L (Jacobs et al. 1985).

The entry of glucose into the glycolytic pathway is controlled by hexokinase (HK), which catalyses the following reaction:



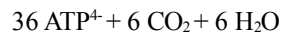
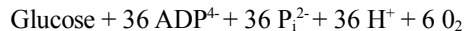
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Plasma hydrogen ion concentration probably does not have an effect on the hepatic release of glucose, though there may be an effect on glucose uptake by the muscle. However, HK activity is strongly inhibited by its product G 6-P (Toews 1966), and the rate limitation at PFK will lead to inhibition of HK as well. The increase in $[\text{G 6-P}]$ also causes the inhibition of GP, which slows the rate of glycolysis (Stanley and Connert 1991).

When the energy requirement is low the majority of the end product is pyruvic acid, which can be utilised aerobically. With a high-energy demand from intense exercise, the rate of glycolysis is increased and there is

an excessive formation of pyruvic acid. The pyruvic acid cannot all be taken into the citric acid cycle resulting in the mass action conversion to lactate, catalysed by LDH (von Duvillard 2001; Juel 2001).

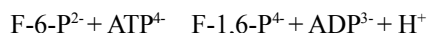
Aerobically, ATP is regenerated through oxidation:



The pK of the reactants is between 6.5 and 6.9 with only small changes in ion concentrations.

Organic acids are both substrates and products of several important reactions. Lac^- is the link between anaerobic glycolysis and severe ionic imbalances during maximal exercise (Sejersted 1992). The hydrolysis of ATP and the production of Lac^- increase the free concentration of protons (Hochachka and Mommsen 1983). The resultant build up of Lac^- can drop the pH of equine muscle from resting values of 7.0 to 6.2 with high intensity exercise (Hyypä et al. 1988). The increased $[\text{H}^+]_i$ has a wide physiological range for the functioning of the muscle's metabolic and contractile processes; a drop from a pH of 7.1 to 6.2 is related to an increase in $[\text{H}^+]$ from 79 to 630 nEq/L.

Phosphofructokinase (PFK) is the key rate limiting enzyme in glycolysis and catalyses the following reaction:



Studies by Jones and colleagues (1977) have shown that acidosis is associated with lower plasma $[\text{Lac}^-]$, suggesting the inhibition of glycolysis as the cause. Sutton and co-workers (1981) performed similar experiments and found the impairment of both glycolysis and Lac^- efflux to be present in the acidic state. They also found evidence to support the theory that changes in muscle glycolytic intermediates were consistent with an inhibition of PFK in acidosis at exhaustion. However, the increasing $[\text{H}^+]$ does not appear to limit glycolytic flux at the rate limiting enzyme PFK as its inhibition can be reversed by increased concentrations of several glycolytic intermediates, despite a high $[\text{H}^+]$ (Jones and Heigenhauser 1992). Studies of humans at maximal exercise show that PFK activity is limiting where $[\text{H}^+]_i$ has increased to 300 mEq/L or more (Jones and Heigenhauser 1992). The increases in $[\text{H}^+]$ have profound effects on the kinetic and structural organisation of the enzyme (Trivedi and Danforth 1966).

Lactate accumulation during high intensity exercise increases muscle acidification by increasing the $[\text{H}^+]_i$.

With intense activity a limited muscle mass may result in a cellular $[\text{Lac}^-]$ greater than 40 mmol/L of cell water and an accompanying decrease in pH of about 0.5 pH units, which may impair muscle function (Juel et al. 1990; Fitts 1994). The increasing $[\text{H}^+]$ has also been thought to limit maximal exercise, however, considerable doubt exists that increased acidification is the proximate limiting factor to metabolic regulation for muscular contraction (Jones and Heigenhauser 2002). The interrelationships between increasing $[\text{H}^+]$, metabolic changes and a decrease in maximal contraction strength may be explained through numerous closely related mechanisms, though currently the specific limiting cause cannot be identified (Jones and Heigenhauser 2002). Westerblad and colleagues (2002) have suggested that inorganic phosphate (P_i) is the major cause of muscle fatigue.

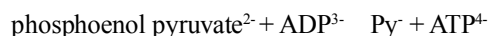
The availability of oxygen doesn't limit oxidative phosphorylation at the beginning of anaerobic exercise (Jones and Heigenhauser 1992) but as the exercise intensity rises and increases energy requirements the available PCr^{2-} stores are used up and glycolysis assumes a greater role in the energy production (Cheema-Dhadli et al. 2002). The level of ATP supply is set by the interaction between glycolysis and oxidative phosphorylation. Its supply then sets the highest steady-state ATP synthesis flux determining the sustainable work level by the exercising muscle (Conley et al. 2001).

To limit ATP supply to within the muscle's oxidative capacity, an accumulation of H^+ s may inhibit a rise in the signal activating oxidative phosphorylation creating a high glycolytic flux (Conley et al. 2001). The limits to ATP production in anaerobic glycolysis have been thought to be how fast glycolysis can be activated and the extent that the accumulation of metabolic intermediates is tolerated (Fitts 1992). However, there is no single limiting factor restricting the continuation of high intensity exercise (Conley et al. 2001).

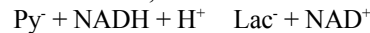
Pyruvate

Newsholme and Start (1977) found the activity of pyruvate kinase (PK) to be greater than that of PFK, and pyruvate (Py) may now be considered the most "influential" of all glycolytic energy metabolites (Jones and Heigenhauser 1992). Pyruvate's influence is exerted on glycolysis, acetyl CoA, lactate, oxaloacetate, and amino acids. However, there is no evidence available regarding the effects of increases in $[\text{H}^+]$ on Py (Jones and Heigenhauser 1992).

The following reaction is catalysed by pyruvate kinase (PK):



The activity of the near equilibrium enzyme lactate dehydrogenase (LDH) is influenced differently in different muscle fibre types. However, its activity is high in all the fibre types, indicating that it is generally dependent on mass action. Glycolytic muscle fibres favour the forward reaction but the reaction is reversed, depending on the NAD/NADH and Py/Lac^- ratios and H^+ , in oxidative fibres:



The conversion of pyruvate and NADH to lactate and NAD in the cytoplasm is catalysed by LDH. The creation of lactate actually consumes a proton and so it can be argued that the term, "lactic acidosis", and its concept are inaccurate. The LDH reaction is actually functioning as a sink for protons and is not contributing to intramuscular acidosis and also is useful for the regeneration of NAD^+ (Robergs 2001).

According to the mass action equation, the $[\text{Lac}^-]$ will always be at least ten times greater than the $[\text{Py}^-]$ and also that increased $[\text{H}^+]_i$ will tend to increase $[\text{Lac}^-]$ by encouraging the forward reaction. The reaction is important when ATP turnover is very high as it aids in maintaining $[\text{NAD}^+]$ for oxidative phosphorylation in fully oxygenated muscle (Jones and Heigenhauser 1992).

There is a strong probability that pyruvate dehydrogenase (PDH) is a key indicator influencing the choice between the utilisation of fat or carbohydrate (CHO) as a fuel and also as a regulator of $[\text{Lac}^-]$ (Jones and Heigenhauser 1992). PDH is a mitochondrial multienzyme complex that catalyses the conversion of pyruvate to acetyl-coenzyme A and regulates entry of CHO into tricarboxylic acid cycle for oxidation (Spriet and Heigenhauser 2002). The activation of PDH during exercise is proportional to the relative aerobic power output and is regulated by increases in $[\text{Ca}^{2+}]$, free $[\text{ADP}]$, and $[\text{Py}^-]$ in human muscle (Spriet and Heigenhauser 2002).

With aerobic exercise pyruvate and NADH increase the flux through LDH, a near equilibrium enzyme. There is little Lac^- production, as it equals the lactate release plus the lactate reconversion to pyruvate in the steady state, and the PDH and shuttle system enzymes metabolise the majority of the substrates (Spriet et al. 2000). The $[\text{Lac}^-]_i$ accumulates because the rate of pyruvate production exceeds the rate of its metabolism by PDH, and because the rate of trans-sarcolemmal Lac^- transport is lower than the rate of pyruvate to Lac^- conversion by LDH (Lindinger 1995). This accumulation creates an alkalinising effect which counteracts the acidifying effects of the increased $[\text{Lac}^-]_i$ and decreased $[\text{K}^+]_i$ (Lindinger 1995).

Increases in $[\text{H}^+]_i$ and altered enzyme activity are

probably linked in a number of ways, however, the ones involved in the more dominant roles has not been determined because of the difficulty in controlling all the experimental variables. Increased $[H^+]_i$ decreases both glycogenolytic (phosphorylase) and glycolytic (PFK) activities (Fitts 1994). Jones and Heigenhauser (1992) proposed that one way the $[H^+]_i$ indirectly influences enzyme activity is with the inhibition of glycogen phosphorylase (GP) and hexokinase (HK) by the glucose 6-phosphate that accumulates when PFK is inhibited. With increasing work rates glycolysis becomes more prevalent as an energy system and there is an increased pyruvate production, perhaps overloading its transporter and other biochemical regulators (Jones and Heigenhauser 1992).

Increased $[H^+]$ and the associated reduction of force are due to decreased excitation-contraction coupling at the level of Ca^{2+} activation of the contractile proteins is another hypothesis for causing muscle fatigue (Donaldson et al. 1978). By competing with Ca^{2+} for the binding site on troponin C, the H^+ could increase fatigue as a greater $[Ca^{2+}]_i$ is then required to produce a given muscle fibre tension (Fuchs et al. 1970). Fabiato and Fabiato (1978) could not counteract the negative effects of the increased $[H^+]$ by increasing the $[Ca^{2+}]_i$, indicating that perhaps the H^+ may modify the tropomyosin molecule rather than directly competing for the binding site. The decreased number of actin-myosin cross bridges formed are a result of decreased binding of Ca^{2+} to the tropomyosin molecule, which slows the contraction rate (Fitts 1994).

Lactate Oxidation

Lactate is not an end waste product of glycolytic metabolism. Once released from the active muscle to the blood it can be taken up by other inactive or less active muscles as well as the heart and the brain, and be oxidized or resynthesized into glycogen (Juel 2001; Jones and Heigenhauser 1992). Lactate oxidation also decreases the systemic acidosis that accompanies increases in extracellular lactate concentration ($[Lac]_e$) (Jones and Heigenhauser 1992). Inactive muscle acts as a sink for lactate and there is an initial rapid uptake reducing its accumulation in plasma. (Lindinger et al. 1990). A further reduction in plasma lactate levels depends on its oxidation within cells:



The simplistic stoichiometry of the reaction suggests that bicarbonate will be reformed, however that doesn't take into account all the variables (Jones and Heigenhauser 1992). The $[HCO_3^-]$ in muscle is low and with an increase of PCO_2 and there is the subsequent diffusion of CO_2 out of the muscle and into venous blood (Johnson et al. 1996). Lactate oxidation is

muscle fibre type dependent. Baldwin and co-workers (1978) found lactate oxidation to be greatest in fast oxidative fibers, intermediate in pure oxidative and lowest in glycolytic fibers (Jones and Heigenhauser 1992).

ACID BASE DETERMINANTS

The Physicochemical Approach

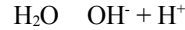
In the 1920s Van Slyke and co-workers found that the total plasma concentration of CO₂ and of non-volatile weak acids affected [H⁺] in physiological solutions. In 1948 Singer and Hastings developed the concept that plasma pH was also affected by the net strong ion charge. In 1978 Stewart recognised this previous work identifying variables affecting [H⁺] and combined it with basic physicochemical principles to describe the behaviour of hydrogen ions.

Stewart based his foundation on three fundamental laws of mass action governing [H⁺] in physiological fluid (Stewart 1981). He realised the physicochemical interactions between the acid-bases independent and dependent variables must recognise the constraints imposed by the conservation of mass, the maintenance of electrical neutrality, and the equilibrium state of weak electrolytes (Stewart 1981, 1983). One of the most important premises of the physicochemical approach is that it is the concentration of the dependent variables (including [H⁺], [OH⁻], [HCO₃⁻], [CO₃²⁻], [HA] and [A⁻]) change when the concentration of the independent variables changes (Stewart 1981, 1983).

This physicochemical approach distinguishes between variables that are capable of acting independently and those concentrations dependent on the equilibrium of all the systems (Jones and Heigenhauser 1992). By defining a series of equations to outline how the dependent variables are affected by the independent variables the systems affecting [H⁺] and [HCO₃⁻] can be described. Each dependent variable can be calculated in terms of the independent variables and the equilibrium constant within each system. The mathematical description of each system allows the calculation of the effect that a change in an independent variable will have on the dependent variables within that compartment. In any physical, biochemical or physiological solution acid-base state can be completely described by the equilibrium between the systems of the independent variables (Jones and Heigenhauser 1992).

Specific Properties of Water

The specific properties of water are important for this approach because H₂O has both a high dielectric constant and an extraordinarily high molar concentration in physiological solution at 55.5 M. These properties cause substances with atoms held together by electrostatic bonds to dissociate when they dissolve but the water dissociation itself is very slight. The dissociation of water is described by the reaction:



The water dissociation reaction reaches equilibrium rapidly so the dissociation of water;

$$[\text{H}^+] \times [\text{OH}^-] = K_w \times [\text{H}_2\text{O}]$$

will have a negligible effect on the water concentration. $K_w \times [\text{H}_2\text{O}]$ can then be considered a constant and rewritten as:

$$[\text{H}^+] \times [\text{OH}^-] = K'_w \quad (\text{Equation 1})$$

where K'_w becomes the ion product for water.

The law of electrical neutrality applies to all solutions and, as H⁺ and OH⁻ are the only ions in pure water, then:

$$[\text{H}^+] = [\text{OH}^-]$$

Which then allows each to be defined as:

$$[\text{H}^+] = \sqrt{K'_w} \quad \text{and} \quad [\text{OH}^-] = \sqrt{K'_w}$$

illustrating that K'_w determines both the [H⁺] and [OH⁻] of pure water.

PCO₂

One of the three independent variables in acid-base chemistry is the [CO₂]. The contribution of CO₂ to the change in [H⁺]_i is usually estimated from the PCO₂ (Stewart 1983). The addition of CO₂ to any aqueous solution and its removal can be summarised by:



Since the amount of dissolved CO₂ can be derived from its solubility constant and the PCO₂, the constants can be described as:

$$K_c = ([\text{H}^+] [\text{HCO}_3^-]) / \text{PCO}_2 \quad (\text{Equation 2})$$

$$K_3 = ([\text{H}^+] [\text{CO}_3^{2-}]) / [\text{HCO}_3^-] \quad (\text{Equation 3})$$

ELECTROLYTES

Electrolytes exist in aqueous solution as charged particles and are considered acids or bases. Cations are positively charged electrolytes and are bases while anions are negatively charged and therefore are acids (Singer and Hastings 1998). The law of maintenance of electrical neutrality requires that there be an equivalent number of cations and anions in solution (Stewart 1983).

Weak Electrolytes

Another independent variable in physiological fluids is the total concentration of weak electrolytes. Weak electrolytes are not fully dissociated in solution. The extent of their dissociation is dependent on the difference between the pH of the solution and their pK (Stewart 1983).

The general dissociation reaction for a weak acid (HA) and conjugate base (A⁻) pair is:



At equilibrium an apparent weak acid dissociation constant (K_A) can be calculated from the law of mass action. HA only partially dissociates into H⁺ and A⁻ ions and so K_A is defined as:

$$K_A = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \quad (\text{Equation 4})$$

The total weak acid/base concentration is designated as [A_{tot}]. A_{tot} can be described as:

$$[\text{A}_{\text{tot}}] = [\text{HA}] + [\text{A}^-] \quad (\text{Equation 5})$$

In resting skeletal muscle the main contributors to A_{tot} are the histidine group of proteins and the metabolites, Cr, P_i, ADP and ATP (Lindinger and Heigenhauser 1990). During high intensity exercise [A_{tot}] increases, contributing to an increased [H⁺]_i (Lindinger and Heigenhauser 1990, 1991). It is assumed that since HA and A⁻ do not take part in any other reactions in the solution, the total amount of substance "A" per litre will remain equivalent to the initial amount.

Weak electrolytes generated by the glycolytic metabolism, including P_i, do not influence [H⁺]_i as they are large charged molecules and mostly unable to cross the muscle membrane (Heigenhauser 1995) so their influence is through intracellular regulation within the muscle (Lindinger 1995). It is estimated that the increases in [A_{tot}] contribute 19% and K_A contributes 7% to the increase in [H⁺]_i (Lindinger 1995).

Strong Electrolytes

The third independent variable is the net strong ion charge. Strong electrolytes dissociate completely in solution, which means they are always in their ionic forms, and can have no buffering effect (Constable 1999). They have a K_A that is far removed from that of H₂O.

The net strong ion charge is represented by what Stewart labelled the Strong Ion Difference (SID). The sum of the completely dissociated cations is usually

greater than the sum of completely dissociated anions, so the strong ion difference has a positive charge (Constable 1999):

$$[\text{SID}] = [\text{strong cations}] - [\text{strong anions}]$$

$$[\text{SID}] = ([\text{Na}^+] + [\text{K}^+]) - ([\text{La}^-] + [\text{PCr}_2^-] + [\text{Cl}^-])$$

The principal strong ions in muscle are sodium (Na⁺), potassium (K⁺), Cl⁻, PCr₂⁻, and Lac⁻, which contribute to SID through biochemical reactions (Colley and Marr 1998; Lindinger et al. 1994). The most important acidifying effects are caused by decreased [K⁺]_i and increased [Lac⁻]_i, while decreases in [PCr₂⁻] exert an alkalinising effect counteracting the acidification (Lindinger 1995).

Continuing from Equation 1, the law of electrical neutrality defines the following equations:

$$[\text{H}^+] \times [\text{OH}^-] = K'_w$$

and

$$[\text{Na}^+] + [\text{K}^+] + [\text{H}^+] - [\text{Cl}^-] - [\text{La}^-] - [\text{PCr}_2^-] - [\text{OH}^-] = 0$$

Since $[\text{OH}^-] = K'_w / [\text{H}^+]$

and

$$[\text{Na}^+] + [\text{K}^+] + [\text{H}^+] - [\text{Cl}^-] - [\text{La}^-] - [\text{PCr}_2^-] - K'_w / [\text{H}^+] = 0$$

the strong ions can be combined into a single term to reduce the equation:

$$[\text{SID}] + [\text{H}^+] - (K'_w / [\text{H}^+]) = 0$$

and simplified to:

$$[\text{H}^+]^2 + [\text{SID}] [\text{H}^+] - K'_w = 0 \quad (\text{Equation 6})$$

Other strong ions in muscle include ammonium (NH₄⁺), calcium (Ca²⁺), magnesium (Mg²⁺), and sulphate (SO₄²⁻) (Kowalchuk and Scheuermann 1995). Calcium and NH₄⁺ elicit small changes, and the charges of Mg²⁺ and SO₄²⁻ are thought to offset each other and so they are not used in the calculations. However, even alterations in [Ca²⁺] and [Mg²⁺] and some other strong ions can affect muscle contractility and fatigability (Lindinger 1994, 1995).

Interaction among Systems

By solving the equations describing water dissociation (equation 1), carbon dioxide (equations 2 and 3), weak electrolytes (equations 4 and 5), and strong acids (equation 6), simultaneously in physiological fluid a single quadratic equation can be created. K_w, K_A, K₃ & K_C are the equilibrium constants of water, weak acids, carbonic acid and bicarbonate, with their values

as follows:

1. $K'_w = [H^+] \times [OH^-]$ $K'_w = 4.4(10)^{-14} \text{ (Eq/l)}^2$
2. $K_c = ([H^+] [HCO_3^-]) / PCO_2$
 $K_c = 2.46(10)^{-11} \text{ (Eq/l)}^2 / \text{mmHg}$
3. $K_3 = ([H^+] [CO_3^{2-}]) / [HCO_3^-]$ $K_3 = 6(10)^{-11} \text{ Eq/l}$
4. $K_A = ([H^+] [A^-]) / [HA]$ $K_A = 3(10)^{-7} \text{ Eq/l}$
5. $[A_{tot}] = [HA] + [A^-]$
7. $[SID^+] + [H^+] - [HCO_3^-] - [CO_3^{2-}] - [A^-] - K'_w[H^+] = 0$

Where, equation 7 is from using [SID] as defined in equation 6,

$$[SID] = ([Na^+] + [K^+]) - ([La^-] + [PCr^{2-}] + [Cl^-])$$

And putting it into the law of the maintenance of electrical neutrality so that

$$[\text{strong ions}] - [\text{weak ions}] = 0$$

Combining the previous equations creates the quadratic equation:

(Equation 8)

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A(SID - A_{tot}) - (K_C * PCO_2 + K'_w)\}[H^+]^2 - \{K_A(K_C * PCO_2 + K_w) + (K_3 * K_C * PCO_2)\}[H^+] - (K_A * K_3 * K_C * PCO_2) = 0$$

Where,

$$(9) A_{tot} = ALB (1.23\text{pH} - 6.31) + (Pi (0.309\text{pH} - 0.469) 10) / 30.97$$

(Figge et al. 1991, 1992)

Equation 8 can be used to calculate the effects of changes of the independent variables on the dependent variables (Johnson et al. 1996). The equation illustrates that an increase in $[H^+]$ within a compartment arises is a result of an increase in PCO_2 , a decrease in [SID], an increase in $[A_{tot}]$, or a combination of these changes within muscle (Kowalchuk and Scheuermann 1995).

At low to moderate exercise intensities the cellular mechanisms of ionic regulation and metabolic states are adequate to prevent an acidosis and maintain homeostasis. With higher intensity exercise, however, it is the changes in the independent variables ([SID], PCO_2 and $[A_{tot}]$) that cause changes in $[H^+]$ and other dependent variables (Stewart 1981, 1983).

In muscle, the changes in [SID] contribute more to $[H^+]_i$ than increases in PCO_2 and $[A_{tot}]$ (Heigenhauser et al. 1990). A decrease in [SID] after 30 seconds of high intensity cycling activity was shown by Lindinger (1995) to contribute 62% to the increase in $[H^+]_i$ as a result of increased $[K^+]_i$ and increased $[Lac^-]_i$. An increase in A_{tot} , mainly from P_i and Cr^{2-} as a result of PCr_2^- breakdown, was found to contribute 19% to the increased acidification. A further 7% increase in $[H^+]_i$ was from the apparent proton dissociation constant (K_A) for $[A_{tot}]$. The rest of the increased acidification, 12%, resulted from the increase in PCO_2 (Lindinger and Heigenhauser 1991).

Table 1*: Contributions to Changes in $[H^+]_i$

Variable	Percent
$[H^+]_i$	100
[SID]	62
$[A_{tot}]$	19
K_A	7
PCO_2	12

*From Lindinger 1995, "Effects of individual changes in independent physiochemical variables on muscle $[H^+]_i$ ".

Traditional Views of Acid-Base Balance

The classical interpretation used to describe acid-base equilibrium focused on $[\text{CO}_2]$, CO_2 pressure (PCO_2), base excess and pH (Hasselbalch 1916; Henderson 1908). The pH was used as an overall measure of acid-base status, with PCO_2 as an independent measure of the respiratory component and the extracellular base excess, $[\text{HCO}_3^-]$, as an independent measure of the metabolic component of acid-base balance (Constable 1999). The description was labelled the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}'_1 + \log \left(\frac{[\text{HCO}_3^-]}{S \cdot \text{PCO}_2} \right)$$

Where PCO_2 = carbon dioxide tension,
 K'_1 = apparent equilibrium constant for the Henderson-Hasselbalch equation,
 pK'_1 = negative logarithm of K'_1 ,
 S = solubility of CO_2 in plasma,
 $[\text{HCO}_3^-]$ = bicarbonate concentration.

Disturbances in acid-base balance can be both considered as metabolic and/or respiratory. Metabolic disturbances were considered in terms of 'base excess', which is a calculated number based on the bicarbonate concentration and excludes the influence of a respiratory component (Conley and Marr 1998). The calculation is based on a measured value for bicarbonate that is adjusted with a PCO_2 of 40 mmHg and expressed as a deviance from the normal value of 25 mmol/L (Jones 1987).

The equation also defines four primary acid-base disturbances, an alkalosis or acidosis within the respiratory or metabolic compartments (Constable 1999). Negative base excess would show a metabolic acidosis, which is associated with a decrease in pH or increase in $[\text{H}^+]$. Positive values for base excess would indicate a metabolic alkalosis, or decrease in $[\text{H}^+]$ and increased pH (Conley and Marr 1998). A respiratory acidosis would be reflected in an increased PCO_2 , whereas a respiratory alkalosis would exhibit a decreased PCO_2 (Constable 1999).

Using equation 4 to define K_A as the dissociation constant for the dissociation of a weak acid, HA, into H^+ and A^- , can be written:

$$K_A = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

and rewritten as: $[\text{H}^+] = K_A \left(\frac{[\text{HA}]}{[\text{A}^-]} \right)$

or $[\text{H}^+] = K_A \left(\frac{[\text{acid}]}{[\text{salt}]} \right)$

Taking the negative logarithm of both sides of the equation yields:

$$-\log[\text{H}^+] = -\log K_A - \log \left(\frac{[\text{HA}]}{[\text{A}^-]} \right)$$

Substituting pH for $-\log[\text{H}^+]$, $+\log[\text{A}^-/\text{HA}]$ for $-\log[\text{HA}/\text{A}^-]$, defining pK_A as $-\log K_A$:

$$\text{pH} = \text{pK}_A + \log \left(\frac{[\text{A}^-]}{[\text{HA}]} \right)$$

creating the Henderson-Hasselbalch equation for the dissociation of a weak acid (Carlson 1997).

Proton Buffering

A buffer is made up of a weakly dissociated acid and the salt of that acid. Compounds act as a proton buffer by minimising changes in $[\text{H}^+]$ by binding protons when the proton concentration in solution increases and releasing them when the proton concentration decreases:



Thus, a buffer takes up or releases H^+ s to stabilise the pH, which enhances the capacity for anaerobic exercise performance (Carlson 1997; Abe 2000).

The muscle's buffer capacity is dependent on its fibre type composition and there is a wide variation in ionic composition of each fibre type (Jones and Heigenhauser 1992). Lindinger and Heigenhauser (1991) found that type II glycolytic fibres have higher glycogen concentrations and glycolytic rates than the other fibre types in their rat hind limb study. They also noted that the type II fibres had the largest [SID], probably because of their higher $[\text{K}^+]$. That means that a smaller increase in $[\text{H}^+]$ will correlate with an increase in $[\text{Lac}^-]$ in muscle made up of type II fibres compared with type I muscle having a smaller [SID] (Lindinger and Heigenhauser 1991).

The buffering system includes extracellular and intracellular buffers as well as the carbonate in bone. The main extracellular buffers include the bicarbonate ($\text{HCO}_3^- / \text{H}_2\text{CO}_3$) buffer pair and plasma proteins. Protein, phosphates, and red cell haemoglobin make up the intracellular buffers with phosphates and proteins contributing to the major part of buffering in muscle (Jones and Heigenhauser 1992). Because weak anions remain partially in ionic form at physiologic pH they can be used as a buffer (Colley and Marr 1998). Bone may provide a large amount of storage, with theoretical estimates that it may contribute up to 40% of the buffering capacity during acidosis, however, it is very difficult to measure accurately (Carlson 1997).

Bicarbonate-Carbonate Buffer Pair

The traditional view of acid-base chemistry within the muscle mainly involves intracellular buffering of the

hydrogen ion by bicarbonate (Wassermann and Casaburi 1991). The $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ buffer pair is the most important from both a clinical and physiological standpoint (Carlson 1997). It is important to recognise that bicarbonate is a dependent variable and changes in its concentration are a result of a metabolic acid-base disturbance (Colley and Marr 1998). The enzyme carbonic anhydrase in the red cells facilitates the buffering action of bicarbonate, which can be represented by (Carlson 1997):



Increased plasma hydrogen ion concentration $[\text{H}^+]_p$, causes the equilibrium equation to shift right so that more H^+ combines with HCO_3^- (Colley and Marr 1988). Increased $[\text{H}^+]$ is minimised as most of the excess H^+ s combine with intracellular buffers, primarily haemoglobin (Hb) (Carlson 1997):



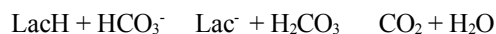
The involvement of both the respiratory and metabolic systems in the bicarbonate buffer system make it unique (Carlson 1997). The CO_2 combines with H_2O creating bicarbonate, which leaves the erythrocyte and enters the plasma in exchange for an extracellular chloride ion creating carbonic acid (Carlson 1997). This enables the CO_2 to be carried to the lungs as HCO_3^- with little change in plasma pH. As the HHb is oxygenated in the lungs, H^+ is released and combines with HCO_3^- to form H_2CO_3 . The carbonic acid is formed back into CO_2 in the lung alveoli and then excreted via respiration (Colley and Marr 1988; Carlson 1997).

The Henderson-Hasselbalch equation applied to this buffer pair becomes (Carlson 1997):

$$\text{pH} = 6.1 + \log \left(\frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \right)$$

Where 6.1 = pK for the $\text{HCO}_3^- / \text{H}_2\text{CO}_3$ buffer pair.

The following reaction shows the link classically proposed on how the lactic acid is buffered by bicarbonate in muscle and blood (Heigenhauser 1995):



High rates of glycolysis create an accumulation of Lac^- in the exercising muscle and related increases in the intramuscular $[\text{Lac}^-]$ that may be between 25 to 32 mmol/L (Bayley 1987; Harris and Snow 1988). Lactate was thought to be buffered by bicarbonate and the close correlation between the arterial $[\text{Lac}^-]$ and $[\text{HCO}_3^-]$ seemed to support this theory (Jones and Heigenhauser 1992). However, studies have shown that there are inconsistencies in the relationship, and that separate mechanisms were causing the efflux of Lac^- and H^+ from muscle.

This Lac^- increase is accompanied by a huge increase in $[\text{H}^+]$ with a horse's muscle pH dropping to as low as 6.2. There are a number of changes accompanying muscle contraction that contribute to an increased $[\text{H}^+]$. Metabolic processes and ion shifts regulate most of the intracellular proton buffering and HCO_3^- plays virtually no part in intracellular buffering (Jones and Heigenhauser 1992). The majority of H^+ s produced in the muscle bind to negatively charged sites on weak acids and bases. Of those, H^+ s primarily bind to intracellular proteins that limit proton buffering to the nanomolar range (Hultman and Sahlin 1980; Rahn and Howell 1978).

Intracellular Buffering

Another buffer system is made up of non-volatile weak acids, composed mainly of carnosine and proteins. The high concentration of proteins and their large number of ionizable groups create the usefulness of this group as buffers (Hyypä et al. 1998). The proteins and peptides rich in histidine residues and with a pK_A value close to the physiologic pH range are the most effective (Hyypä et al. 1998).

The intracellular non-bicarbonate buffering capacity of equines is dominated by carnosine, a histidine-containing dipeptide. Inorganic phosphate, histidine residues in proteins, and organic phosphate compounds also contribute to proton buffering. However, the cellular concentration of these other buffering components may be constrained because their primary physiological roles have nothing to do with buffering (Abe 2000).

Carnosine is a dipeptide containing histidine with a pK_A of 6.38. Equines, and other fast running animals, generally have high carnosine concentrations in comparison with slower mammals. Horses can have as much as ten times the concentration when compared to man (Marlin et al. 1989). Type II fibres have higher carnosine concentrations than type I, which closely correlates the muscle fibre type distribution of equines with their buffering capacity (Dunnnett and Harris 1995; Marlin et al. 1989).

Sewell et al. (1992) found that carnosine accounts for up to 50% of the physico-chemical buffering of H^+ produced by type IIb muscle fibres in the pH range of 7.1-6.5. They also found up to 30% in IIa fibres and 20% in type I when looking at the gluteus medius in thoroughbreds. Type II muscle fibres are especially abundant in muscle of excellent mammalian sprinters such horses (Abe 2000).

The muscle fibre type distribution and carnosine levels vary between breeds. The gluteus medius of quarter

horses was found to consist of about 12% type I, 49% type IIa, 39% type IIb fibres and contain about 39 $\mu\text{mol/gr}$. wet weight of carnosine (Bump et al. 1990). Thoroughbreds and standardbreds were found to have around 16% type I, 60% type IIa and 24% type IIb muscle fibres with 27-31 $\mu\text{mol/gr}$. wet weight of carnosine. Across the breeds muscle carnosine concentration was positively correlated with fast twitch glycolytic fiber percentage and negatively with fast twitch oxidative fiber percentage (Bump et al. 1990).

Enzymes

Enzymes can also work as buffers and the other dynamic buffering system is the reaction catalysed by glutamine synthetase:



In muscle this reaction is limited by the $[\text{NH}_4^+]$ (Blackshear et al. 1975). Glutamine increases after submaximal exercise to fatigue in equines, but the effect of maximal exercise on its concentration is unknown. However, the reaction supplying the NH_4^+ is known to increase during maximal effort by the breakdown of adenine nucleotides (Sewell et al. 1992).

Red Blood Cells

As lactate is transported from muscle into the blood it is buffered in the plasma by its uptake into red blood cells (RBCs). Horses have increased lactate distribution and volume because of the catecholamine-induced mobilisation of erythrocytes from the splenic reservoir into the circulation. The net effect of this 'splenic dump' along with an increased oxygen carrying capacity creates an increased H^+ buffering capacity in the exercising animal (Hyypä et al. 1998).

Decreasing intramuscular acidification in exercising muscle cells is important for prolonging the muscle's capacity for anaerobic work (Carlson 1997). The ability of muscle to reduce its rate of acidification by its buffer capacity may be a key determinant in sprint performance (Hyypä et al. 1998). Other factors influencing $[\text{H}^+]_i$ include extracellular buffering and the PCO_2 within the respiratory system (Carlson 1997). The rate of muscular acidification is seen as a function of the rate of proton formation based on the glycolytic and oxidative capacity of the muscle, its buffer capacity, and the rate of proton and lactate efflux out of the fibres (Hyypä et al. 1998).

LACTATE TRANSPORT

The Lactate Shuttle

Historically Lac^- was viewed as a dead-end waste product that moved across muscle membranes by diffusion (Bonen 2001). Brooks (1988) developed the 'lactate shuttle hypothesis' and established Lac^- as a useful metabolite that can be rapidly transferred among different tissue compartments greatly influencing acid-base balance. The uptake and efflux of Lac^- across the sarcolemma and mitochondrial membrane involve facilitated diffusion (Juel 2001). Lac^- transporters include passive diffusion (Dubinsky and Racker 1978), the H^+/Na^+ exchange protein, the anion exchange system and the monocarboxylate (MCT) carrier (Bonen 2001).

Passive Diffusion

During exercise $[\text{H}^+]$ is less than $[\text{Lac}^-]$ in muscle cells because of the strong ion exchange and the reactions consuming H^+ , including Ck and glutamine synthetase. This gradient increases the efflux of protons from the muscle into the plasma (Bonen 2001) primarily due to physicochemical reactions and the fact that the solvent is water ($\text{H}^+ + \text{OH}^-$) (Juel 2001). The $[\text{Lac}^-]$ increases the proton efflux through passive diffusion (Bonen 2001). Certain hormones, such as catecholamines, appear to increase the transport of lactate as well (Stainsby et al. 1985). At very high $[\text{Lac}^-]$ diffusion could account for a large percentage of the Lac^- movement across the plasma membrane, however, this type of movement out of the muscle cell is inefficient as the lactate anion moves slowly across the plasma membrane (Bonen 2001).

The H^+/Na^+ -Exchange Protein

All animal cells use the H^+/Na^+ -exchanger to remove protons. This carrier is activated by $[\text{H}^+]_i$ and is driven by the Na^+ gradient across the sarcolemma and mitochondrial membranes (Madshus 1988) as well as the pH (Bonen 2001). The Na^+ ions are then combined with the Na^+/K^+ -ATPase and transported out of the cell (Bonen 2001).

The Anion Exchange System

The anion exchanger, or band 3 protein, has a K_m of 3 mM for Lac^- (Cheeseman et al. 1994). The low K_m makes this carrier an effective lactate transporter at the early stage of exercise and exchanges Lac^- with an inorganic ion such as HCO_3^- or Cl^- (Bonen 2001). However, the activity of the anion exchanger is rather low in sarcolemma and probably plays only a minor role in Lac^- transport (Poole and Halestrap 1993).

The Monocarboxylate Carrier

Seventy to 80% of the Lac^- transfer taking place during high intensity exercise involves facilitated diffusion by

a membrane protein called a MCT that co-transporters the Lac^- , along with a H^+ , out of the cell (Stainsby and Brooks 1990; Juel 1997). The MCT system is stereospecific, pH dependent and has an obligatory 1:1 coupling between Lac^- and H^+ fluxes (Bonen 2001; Juel 2001). The MCT is the main carrier of Lac^- into RBCs in horses and the amount of available MCT proteins determines the efficiency of the transsarcolemmal lactate transport and regulates $[\text{Lac}^-]$ in skeletal muscle (Väihkönen and Pösö 1998; Bonen 2001). The MCT can be reversibly blocked by the competitive inhibitor cinnamate and irreversibly blocked by mercury-containing compounds (Juel 2001).

High Lac^- transport levels are typical for athletic animals, including horses (Skelton et al. 1995), but there is a wide individual variation following maximal exercise (Hyypä 1998). Väihkönen and colleagues (1999) found two distinct groups of horses with either high or low MCT activity following trotting races. Pösö et al. (1995) found that the large distribution differences found after trotting races could be explained by the fivefold interindividual variation in activity of the MCT.

MCT Isoforms

Equine skeletal muscle expresses both MCT1 and MCT4 proteins and the proposed roles for those isoforms are based on their distribution in the different fibre types (Bonen 2001; Juel 2001). MCT1 is abundant in oxidative fibres and it is thought that they are specialised for the uptake of Lac^- into cells for oxidation (von Duvillard 2001). The MCT4 is evenly distributed throughout the fibre types and they may also be used for Lac^- efflux from the muscle (Wilson et al. 1998).

The activity of the MCT in equine muscles has not been measured, and there is a wide range of K_m values for lactate efflux in human beings, rats, and mice from 3.5 to 46 mmol (Lindinger et al. 1995). However both isoforms have an identical K_m , though they have different roles in muscle and are regulated separately (von Duvillard 2001).

The close correlation of MCT1 proteins and oxidative muscle fibres matches the higher Lac^- and H^+ transport capacity in those fibres as compared to the more glycolytic ones (Pagliassotti et al. 1990). An increased $[\text{MCT1}]$ increases both cellular Lac^- influx and efflux in muscle (Juel 2001). Various studies on rat hind muscle preparations have shown that Lac^-/H^+ transport capacity in a slow-twitch oxidative fibre is about twice the capacity in a fast-twitch glycolytic fibre (Bonen and McCullagh 1994; Juel et al. 1991). The increased ability to transport both Lac^- and H^+ out of the

oxidative fibres may explain why they are more fatigue resistant compared to the more glycolytic fibres (Juel 2001). Also, the oxidative muscles may need to release their Lac^- at higher rates as they produce it during intense exercise and are recruited for longer time periods (Juel 2001). Because of the increased transport capabilities in oxidative fibres, peak Lac^- production may be greater than has been thought (Juel 2001).

While MCT1 expression is induced aerobically, MCT4 expression is induced by anaerobic stress imposed on the muscle (Juel 2001). The MCT4 density has a large interindividual variation and appears to be independent of muscle fibre type (Juel 2001). There has been high positive correlation found between both the MCT4 and the percentage of fast glycolytic fibres and between MCT4 and LDH activity (Bonen 2001), but a negative correlation between both MCT4 and Lac^- uptake from the circulation and between MCT4 and MCT1 (Bonen et al. 2000). MCT4 proteins have been found in only type II fibres and its expression seems confined to muscle fibres with high glycolytic rates (Pilegaard et al. 1999). The decreased rate of Lac^- transport in glycolytic fibres may relate to a greater retention of Lac^- during recovery from exercise. The retained Lac^- is then more likely to be resynthesized into glycogen (Pagliassotti et al. 1990).

MCTs are important in the regulation of pH in skeletal muscle (Juel & Halestrap 1999). By reducing concentration of a strong acid anion $[\text{Lac}^-]$, and so increasing $[\text{SID}]$, muscle pH must be increased. In rat muscle studies Juel (1995) found that the MCT, as compared to other Lac^- transporters, has the greatest capacity for mediating H^+ efflux from the muscle cells. A number of experiments have found that the Lac^-/H^+ cotransport system probably has the highest H^+ removal from muscle during intense exercise (Juel et al. 1990; Pilegaard et al. 1999; Bangsbo et al. 1997).

SUMMARY

Traditionally it was thought that the accumulation of Lac^- during high intensity exercise was a result of an oxygen limitation within the muscle cell. The term 'anaerobic' or 'lactate' threshold was given to indicate the point where exercise intensity reaches an abrupt increase in $[\text{Lac}^-]_p$ (Wasserman et al. 1986). However, lactate is also created and utilized under fully aerobic conditions (Brooks 2001) and there seems to be more of a moderate rise in plasma lactate levels (Myers and Ashley 1997). The formation of lactate depends on several factors, only one of them being oxygen availability (Brooks 2001).

Lactate is a metabolic intermediate that can be oxidized or utilized as energy. The mandatory coupling of Lac^-

and H^+ increases intramuscular acidification that may have many effects resulting in the limitation of high intensity exercise. During experiments where the acid-base variables were manipulated during exercise inconsistencies appeared in these relationships (Jones et al. 1977). Problems also appeared in animal experiments where $[Lac^-]$ changes were not related to changes in $[H^+]$ (Jones and Heigenhauser 1992). Subsequent studies indicated that a variety of conditions were contributing to increased $[H^+]$ with exercise (Stainsby and Eitzman 1988, Heigenhauser et al. 1990).

Using a multifactorial theory to explain force reduction during high intensity has $[H^+]$ affecting Lac^- directly through the inhibition of key glycolytic enzymes as well as indirectly through inhibiting the contractile process (Bonen 2001). Heigenhauser and colleagues (1990) estimated the relative contributions of the different factors from studies with exercising humans. They found that increases up to 30% may be due to increases in PCO_2 , 40% or more may be due to reductions in $[SID]$ and 30% due to changes in $[A_{tot}]$ and K_A (Jones and Heigenhauser 1992).

A major problem with the Henderson-Hasselbalch approach is that in principle it is much more descriptive than mechanistic (Jones 1987, Stewart 1981) and the greatest advantage of the physicochemical approach over the traditional is in its quantitative assessment (Constable 1999). A limitation with the traditional approach is that the equation can only be accurately applied to ruminant plasma at approximately normal T, pH, protein concentration, and sodium concentration (Constable 1999). The physicochemical approach permits a better evaluation of a greater range of acid-base disorders as well as allowing greater understanding of acid-base physiology (Aguilera-Tejero 2000). It also brings us past the conventional descriptions of acid-base in terms of the CO_2 system as the descriptions of base excess and deficit are limited do not work intramuscularly (Johnson et al. 1996). By acknowledging the numerous variables involved in acid-base balance, bicarbonate becomes only one of the many factors influencing lactate concentration (Johnson et al. 1996).

The existence of the Lac^-/H^+ cotransporter reduces the increasing $[Lac^-]$ during activity in the active muscles and facilitates its uptake in other fibres (Juel 2001). From their study Tonouchi and co-workers (2002) found that the contraction-induced increases in Lac^- transport, occurring at high $[Lac^-]$, may be attributed to changes in the intrinsic activity of MCT transporters. The internal pH is regulated by the H^+/Na^+ exchanger, which serves as a safety system against any major changes (Juel 2001). It seems that the transport systems not involving Lac^- regulate pH at rest, and

contribute to its fine adjustment, and that the MCTs are the main transporter for the large Lac^- productions with intense exercise (Juel 2001).

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